Effects of Irradiation on Holmium AcetylAcetonate Microspheres

Imke Boekestijn
Reactor Institute Delft
imkeboekestijn@hotmail.com

SRC 2019, December 5, 2019, The Netherlands.

ABSTRACT
The effects of irradiation on recently developed holmium acetylacetonate (HoAcAc) microspheres have been investigated by exposing HoAcAc microspheres to different irradiation conditions (types and doses) and temperatures. An accurate and detailed characterization of the chemical and physical properties of the HoAcAc microspheres before and after exposure to different conditions enabled the determination of the influence of neutron and/or gamma irradiation on the HoAcAc microspheres. The most important observation is that irradiation has a damaging effect on the microspheres, which occurs mostly at chemical level. Furthermore, results reveal a positive influence of the temperature in combination with irradiation on the microspheres.

Keywords
Microspheres, holmium, neutron irradiation, selective internal radiation therapy.

INTRODUCTION

Annually, around 700,000 people die from primary liver cancer (American Cancer Society) and a similar number of patients die from metastatic malignancies in the liver. The treatment options for tumors in the liver are still limited, therefore the mortality-over-incidence ratio is high (0.93). Current treatments are mostly used to control tumour growth and relieve the symptoms, whereas, treatment with radioembolization has shown very high response rates. Intra-arterial radioembolization combines embolization and radiotherapy through the use of microspheres containing radioactive isotopes. In contrast to normal liver tissue, which derives most of its blood supply from the portal vein, hepatic malignancies receive their blood supply almost entirely from the hepatic artery. Therefore, microspheres are injected into the hepatic artery and lodges into the vascular bed of the liver. Their size, 20-50 µm, prevents them from returning into the blood flow. Through the years different isotopes have been tested for radioembolization. One of the most commonly used isotopes for this purpose is the beta-emitting isotope yttrium-90. Microspheres containing yttrium-90 are commercially available under the brand names TheraSphere and SIR-spheres. However, due to the lack of primary gamma-rays the imaging options are very limited. As a result, microspheres loaded with holmium-166 were developed. Holmium-166 emits beta-minus (β^-) particles, which are suitable for therapy, as well as gamma-rays. The energy of the emitted gamma-rays is ideal for nuclear (SPECT) imaging. Beside the convenient radiation properties of holmium-166, holmium is also highly paramagnetic and has a high linear attenuation coefficient allowing visualization by magnetic resonance imaging (MRI) and X-ray computed tomography (CT) [1,2,3].

Currently, holmium-166 loaded microspheres are successfully used as a treatment for liver cancer. These microspheres contain 19 wt% of holmium which is incorporated in a matrix of poly(lactic acid). Holmium-166 microspheres are commercially available under the brand names QuiremSpheres® and QuiremScout® and are produced by Quirem Medical (Deventer, The Netherlands). To extend the applications of holmium-166 microspheres, a higher specific activity per sphere is required. To obtain this higher specific activity, either microspheres with a higher holmium content need to be developed or the irradiation time needs to be increased. However, the irradiation time can lead to radiation damage [4]. The stability of microspheres for radioembolization is of utmost importance to ensure their clinical efficacy and safe use. However, the effects of neutron irradiation on the recently developed holmium acetylacetonate microspheres is yet unknown.

In this project, the effects of the irradiation conditions (types and doses), volume of sample irradiated and temperature on the microspheres are investigated. Different irradiation types and doses containing gamma and/or neutron irradiation will be achieved by varying the time span of the irradiations done in either the Hoger Onderwijs Reactor (HOR) or the gamma photon source GC-220 cobalt-60, operational at the Department of Radiation Science and Technology of the Delft University of Technology, The Netherlands. Furthermore, the influence of temperature on the microspheres are tested using an oven. The damages on the microspheres are defined by decrease of acetylacetonate content, diversity in water, holmium content and visual inspection of surface properties. An extensive characterization of the microspheres' composition and physical condition provides the necessary information to conclude on the effects of irradiation on holmium acetylacetonate microspheres.

METHODS

Preparation of HoAcAc microspheres
For this study a solvent evaporation method was used to

prepare HoAcAc microspheres (HoAcAc-MS). These developed microspheres contain holmium-165 and acetylacetonate. For this purpose, crystals made of holmium acetylacetone were prepared. Briefly, an aqueous solution of acetylacetone was combined with a holmium chloride solution and the pH of this solution was adjusted to 8.5 by adding an ammonium hydroxide solution. In order for the holmium acetylacetonate to crystallize, the solution was put aside for 24 hours. The formed HoAcAc crystals were subsequently collected and washed three times with water. The washed crystals were dried for 64 hours in a vacuum oven. These crystals have the property that they are insoluble in water but readily soluble in organic solvents. After obtaining the HoAcAc crystals, 10 grams of HoAcAc crystals were dissolved in 186 grams of chloroform. Chloroform is an organic solvent, therefore, HoAcAC crystals dissolve in chloroform. Since either chloroform or HoAcAc are insoluble in water, the HoAcAc will remain in the chloroform droplets. To keep the emulsion droplets stable, the chloroform solution was added to an aqueous PolyVinyl Alchohol (PVA) solution in a double baffled beaker and continuously stirred at 300 rpm (Figure 1, step 1). The PVA forms a thin layer around the droplets keeping them stable and in a spherical shape. The chloroform was evaporated at temperature of 27 °C due to the use of a water bath and a constant nitrogen flow of 12 L/min. After evaporating for 72 hours, the microspheres were collected and washed three times with water. To obtain the desired sizes, the microspheres were sieved through a 50 µm with the use of an electronic sieve vibrator to discard microspheres with sizes above 50 µm and collected in a sieve of 20 µm to discard microspheres smaller than 20 µm. The microspheres were then dried in a vacuum oven for 72 hours.

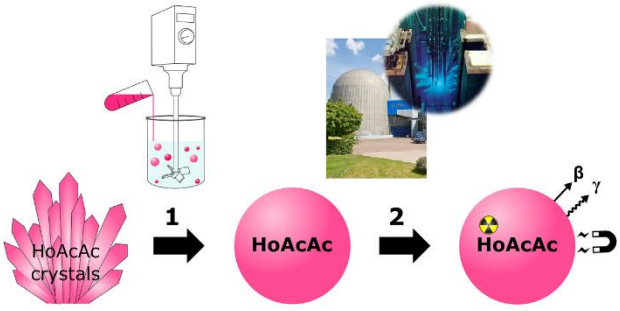
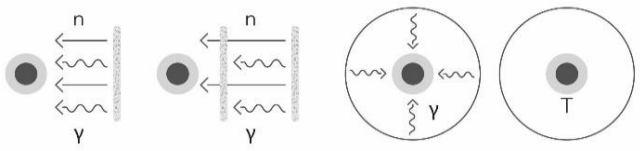


Figure 1. Schematic overview of the preparation of radioactive HoAcAc microspheres. 1) Microspheres are made by an emulsion of HoAcAc crystals and chloroform followed by solvent evaporation. 2) The microspheres are neutron activated in a nuclear reactor converting ^{165}HoAcAc-ms into ^{166}HoAcAc-ms [4].

Irradiation of HoAcAc microspheres

Radiation can be induced in a nuclear reactor with the use of a neutron activation technique. The basic principle of the neutron activation technique lies with bombarding the target isotope, in this case the holmium-165 in the HoAcAc microspheres, with neutrons. The nuclei of the stable holmium-165 captures free low-energy neutrons, resulting in heavier nuclei entering the excited state and turning the isotope possibly unstable with its own half-life and radiation properties (Figure 1, step 2). After neutron activation of holmium-165, not only holmium-166 with a half-life of 26.83 h is formed, but also other isotopes, e.g. holmium-166m with a half-life of 1200 y, is formed. Either holmium-166 and holmium-166m decay by emitting β^-- and γ-radiation. The neutron activation of HoAcAc microspheres has an effect on the microspheres. To determine the nature of this effect, the microspheres have been irradiated in different irradiation facilities of the Hoger Onderwijs Reactor as well as in a Co-60 GC-220 facility. The HOR is equipped with several irradiation positions used for neutron irradiation and activation analysis, shown in Figure 2. In this study, the majority of the irradiations of the microspheres were done in the A8 position with a theoretical dose of 188 kGy/h, of which 171 kGy/h is gamma radiation, and a thermal flux equal to $3.71 \cdot 10^{16}$ n $\cdot$ m^{-2}s^{-1}, epithermal flux of $5.2 \cdot 10^{14}$ n $\cdot$ m^{-2}s^{-1} and a fast flux of $1.95 \cdot 10^{15}$ n $\cdot$ m^{-2}s^{-1}. Different amounts of ^{165}HoAcAc-ms (150, 300 and 600 mg) were irradiated in the A8 position for 1, 2, 4 and 8 hours obtaining a gamma dose of 171, 342, 684, 1368 kGy and a total dose of 188, 376, 752, 1504 kGy respectively. One irradiation was done in a flexible irradiation facility (FlexBeFa) located in the C8 position with a theoretical total dose rate equal to 60 kGy/h, a thermal flux of $5.68 \cdot 10^{16}$ n $\cdot$ m^{-2}s^{-1}, epithermal flux of $1.14 \cdot 10^{15}$ n $\cdot$ m^{-2}s^{-1} and a fast flux of $3.40 \cdot 10^{15}$ n $\cdot$ m^{-2}s^{-1}. 300 mg of ^{165}HoAcAc-ms were irradiated in the C8 position over a time span of 10 hours receiving a total dose of 600 kGy. Due to the lead material of the FlexBeFa it decreased the gamma radiation and therefore, it was expected to reduce the damages on the microspheres caused by gamma radiation. The Co-60 GC-220 facility uses the decay of cobalt-60 as a constant source of gamma radiation. This facility was used to examine the influence of gamma radiation on the microspheres. Hereby, the microspheres were exposed to a gamma dose similar to the HOR gamma doses. The current dose rate of the Co-60 GC-220 facility is equal to 0.236 Gy/s. An amount of 300 mg of ^{165}HoAcAc-ms were irradiated in the GC-220 over a time span of 52, 120, 288 and 624 hours, receiving a gamma/ total dose of 44.4, 102.5, 245.9 and 532.8 kGy respectively. All irradiations were performed in a München rabbit. Furthermore, the influence of temperature on the microspheres was examined for temperatures equal to the ones in the irradiation position A8, 105 °C, and in the irradiation position C8, 47 °C. All experimental set-ups are shown in Figure 2.



	A8	C8	GC - 220	Oven
Temperature [°C]	105	47	20	105/47
Dose rate [kGy/h]	188	60	0,85	-

Figure 2. A schematic view from above of the experimental set-ups, where the black filled circle simulates a microsphere and the gray circle illustrates the temperature. Furthermore, the temperature and dose rate per facility is mentioned.

Characterization of microspheres

The HoAcAc crystals and HoAcAc-MS (before and after irradiation) were characterized for their acetylacetonate content by making use of an Alliance HPLC system equipped with a C18 column (XSelect CSH C18 3.5m 4.6×150mm, Waters) at 40 °C. Around 15 to 20 mg of samples were dissolved in 5 mL of pure methanol and 5 µL of this solution was injected into the column. EDTA-titration was used to determine the holmium content in the crystals and microspheres. For the titration, 1.861 grams of EDTA was dissolved in 500 mL water. The samples were dissolved in 10 mL 1% nitric acid solution subsequently 5 grams of methenamine and approximately 40 g of water are added. The water content is determined with the use of a Karl Fischer Coulometer (831, Metrohm). Samples were dissolved in pure methanol to release the water and the water concentration in this solution was measured by injecting 50 µL of the solution. Furthermore, JOEL's SEM JSM-IT100 was used to determine the surface properties of the HoAcAc-MS. Hereby, the voltage was set on 1.5 kV and the following magnifications were used, 100x, 400x and 1000x. The size of the ^{165}HoAcAc-MS was measured with a Beckman Coulter Multisizer 3. Around 5-10 mg of microspheres incubated in 1 mL of water was added to the electrolyte and measured for a time span of 90 seconds. Moreover, the activity of the microspheres after neutron activation in the A8 position was measured with a doses calibrator after a decay time of approximately 2 weeks. The value of the activity right after irradiation (A(0) in Bq) is calculated with the use of Equation 1, where λ is a constant depending on the half-life $T_{1/2}$ of the isotope and is equal to $\ln(2)/T_{1/2}$ and A(t) is the current activity of the isotope.

$$A(t) = A(0) \cdot e^{-\lambda t} \quad (1)$$

Furthermore, the activity after the neutron activation was additionally calculated with de formula given in Equation 2, where *y* is equal to the amount of targeted isotopes.

$$A_{irr}(t_{irr}) = y\left(1 - e^{-\lambda t_{irr}}\right) \quad (2)$$

RESULTS AND DISCUSSION

Characterization of HoAcAc microspheres

The HoAcAc crystals and HoAcAc-MS were chemically characterized on their acetylacetonate, holmium and water content. The HoAcAc-MS were also characterized on their size and surface properties. The mean size and chemical composition are given in Table 1. Comparing the acetylacetonate content of the crystals with the microspheres, it shows a decrease of 14% weight percentage of acetylacetonate throughout the production process of forming new molecular arrangement of microspheres. Furthermore, the structure of the HoAcAc microspheres contains 2 water molecules and therefore the water content should be around 7.5 % which is confirmed with the measured water content. Moreover, the mean size of the microspheres is 34.31 ± 5.6 µm which is acceptable within the standards of 20-50 µm. The microphotographs made with the scanning electron microscope showed no visual abnormalities of the microspheres, shown in Figure 3A.

Characterization after irradiation

The neutron activated HoAcAc-Ms, in either C8 or A8 position of the HOR, are chemically characterized on acetylacetonate content and water content. The HoAcAc-Ms irradiated in the Co-60 GC-220 are also characterized on their holmium content and show a maximum decrease of 3.14 ± 0.31%. A decrease of the acetylacetonate content from 51.5 ± 4.2 to 37.8 ± 0.1%, 33.2 ± 1.8% and 18.7 ± 0.6 % was observed after irradiation of the microspheres

Table 1. Characteristics of HoAcAc crystals and HoAcAc-MS with the weight percentages of the acetylacetonate content, holmium content, water content and mean size. The associated uncertainties are based on the standard deviation.

Sample	AcAc [wt%]	Ho [wt%]	H_2O [wt %]	Mean Size [µm]
HoAcAc crystals	64.9 ± 2.5	33.3 ± 0.5	3.83 ± 0.20	n.a.
HoAcAc-MS	51.5 ± 4.2	44.6 ± 0.2	7.75 ± 0.61	34.31 ± 5.6

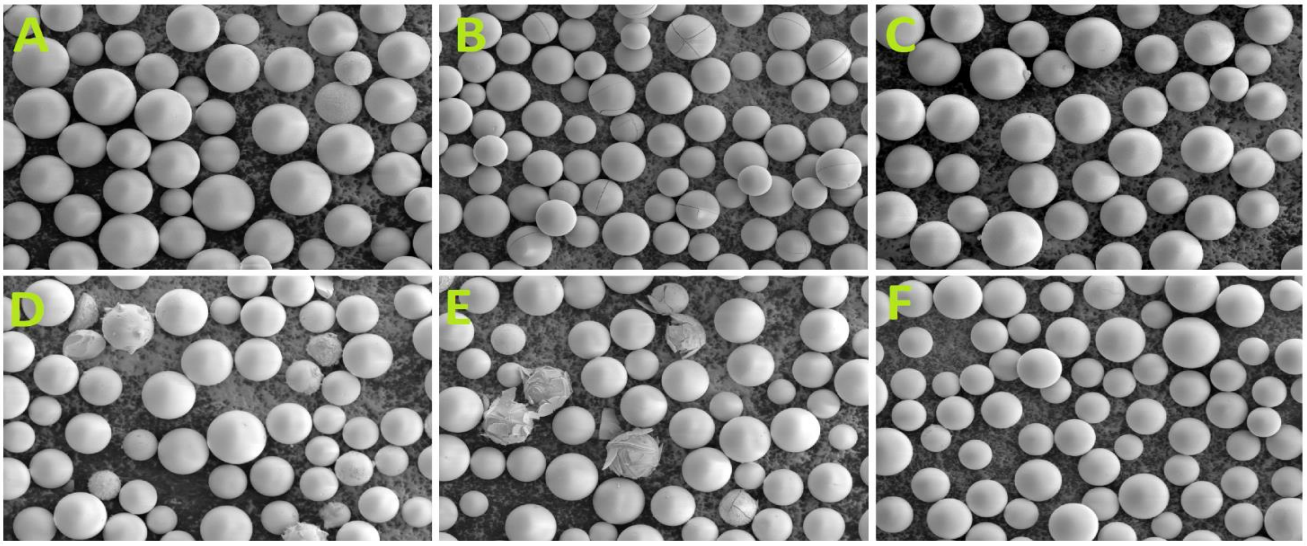


Figure 3. Scanning electron microphotographs of (A) the microspheres before irradiation, (B) the microspheres irradiated in the Co-60 GC-220 for a dose of 532.8 kGy, (C) microspheres irradiated in the C8 position of the HOR for a total dose of 600 kGy with a sample amount of 300 mg and (D, E and F) microspheres irradiated in the A8 position of the HOR for a total dose of 188 kGy with sample amounts of 150, 300 and 600 mg respectively.

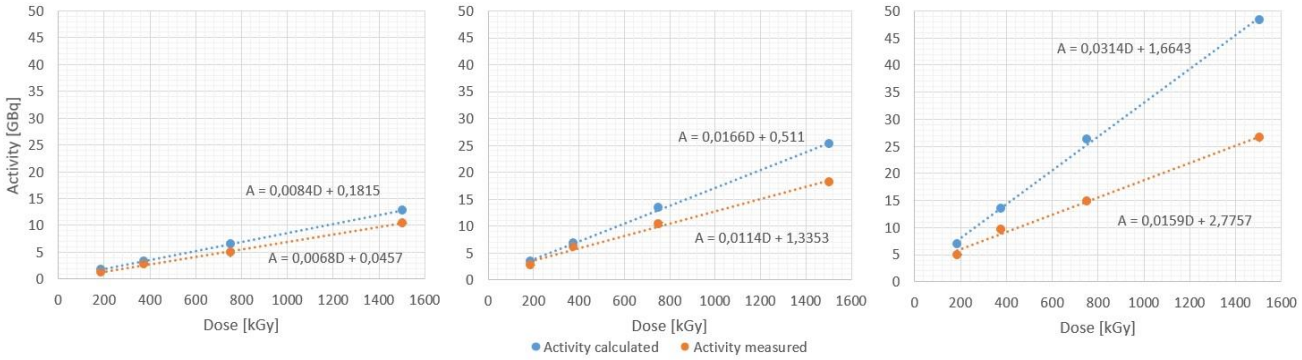


Figure 4. Calculated activity compared to the measured activity of the samples with mass 150 mg (left), 300 mg (middle) and 600 mg (right) as a function of dose.

with 1504 kGy (A8, 105 °C), 600 kGy (C8, 47 °C) and 532.8 kGy (Co-60 GC-220, room temperature) respectively. These results reveal a positive influence of the temperature in combination with irradiation on the microspheres suggesting a possible annealing process in the microspheres due to a heating treatment throughout the irradiation. Furthermore, measurements of the water content showed no significant change. Visual inspection of the surface properties shows an increased damage due to gamma irradiation. The microphotograph of the irradiation done in the Co-60 GC-220 for a dose of 532.8 kGy at room temperature (Figure 3B) showed damages while the microphotograph of the irradiation done in the C8 position in the HOR for a dose of 600 kGy at 47 °C showed no damages (Figure 3C). Moreover, the microspheres irradiated in the A8 position show some surface damages in the samples irradiated with a doses of 188 kGy and sample masses of 150 and 300 mg (Figure 3D and 3E). These damages are not observed in the samples irradiated with 600 mg (Figure 3F), probably due to a self-shielding effect of the holmium in the microspheres. During irradiation in the HOR, the radiation was originating from one direction and as a consequence, holmium of the microspheres located closer to the irradiation source absorbed the neutrons from the radiation, shielding the microspheres located more to the back of the irradiation vial. This shielding effect is substantiated by the values of the measured activity compared to the calculated activity shown in Figure 4. The calculated activity is continuously higher than the measured activity. However, the difference increased with increasing the sample amount. At last, no significant change either chemically or visual was found in the temperature analysis, concluding microspheres are resistant to temperatures up to 105 °C.

CONCLUSION

An accurate and detailed characterization of the microspheres before and after exposure to different irradiation conditions (type and doses) and temperatures show that irradiation damages the holmium acetylacetonate microspheres which mostly occurs on a chemical level. This is shown in a decrease in acetylacetonate content as well as a small decrease in holmium content. Furthermore, these results reveal that an increasing temperature in combination with irradiation protects the acetylacetonate molecule. Dose rate, spectrum of gamma photons and temperature differ between the irradiation facilities, however, only the temperature has an influence significant enough to possibly cause a lesser acetylacetonate decrease in the microspheres while irradiated for a higher doses. When investigating the temperature influence separately, no damages are found in either the chemical or surface properties. The positive effect of the temperature in combination with irradiation could be explained by the annealing process. Moreover, a so called self-shielding effect occurs while irradiating the microspheres in the HOR. During irradiation in the HOR, the radiation was originating from one direction. Therefore, the microspheres located closer to the irradiation source received more radiation, shielding the microspheres located more to the back of the irradiation vial. As a consequence, the observed activities from the irradiated microspheres were lower than the calculated activities. Future studies will focus on the annealing process and improving the amount of activity after neutron activation.

ROLE OF THE STUDENT

This paper is based on the authors' thesis submitted for her graduation project as an undergraduate student under supervision Alexandra Arranja. The topic was proposed by Antonia Denkova. The production of the microspheres and obtaining the results have been done by the student under supervision. Furthermore the irradiations were done by the staff of the Hoger Onderwijs Reactor.

REFERENCES

1. Quirem. *Quirem medical.* Available at: http://www.quirem.com [Accessed Feb. 2018]
2. American Cancer Society. *Treating liver cancer.* Available at: https://www.cancer.org/cancer/liver-cancer/treating.html [Accessed Feb. 2018]
3. Bult, W., Seevinck P.R., Krijger, G.C., Visser T., Kroon-Batenburg, L.M.J., Bakker, C.J.G., Hennink, W.E., van het Schip, A.D. and Nijsen, J. F.W. *Microspheres with Ultrahigh Holmium Content for Radioablation.* Pharmaceutical Research (2009), 26.
4. Nijsen, J.F.W., van het Schip, A.D., Hennink, W.E., Rook, D.W., van Rijk, P.P. en de Klerk, J.M.H. *Advances in Nuclear Oncology: Microspheres for Internal Radionuclide Therapy of Liver Tumours.* Current Medicinal Chemistry (2002), 9, pp. 73-82.
5. Arranja, A.G., Hennink, W.E., Denkova, A.G., Hendrikx, R.W.A, Nijsen, J.F.W. *Radioactive holmium phosphate microspheres for cancer treatment.* International Journal of Pharmaceutics (2018), 548, pp. 73-8